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Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Adult soft tissue sarcomas—Treatment and monitoring

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Major advances have been achieved during the past decades in the management of localized STS. Amputation surgery has become infrequent, local failure is rare, and the interest has been increasingly focused on quality of life in comparing different combined modality treatment approaches. The treatment schedule in this study yielded acceptable local control, but was accompanied by a high rate of late complications. This was probably in part due to the high radiation dose per fraction. With good radiotherapy technique, however, and small treatment volumes some patients with extremity STS retained excellent functional results, which underscores the need for centralization of these patients.

Postirradiation sarcomas are rare, but comprise an important subgroup of sarcomas both from a theoretical and practical point of view. The prognosis in this population based series compares favourably to previous reports, and emphasizes the need for life-long follow-up of cancer patients.

Haematogenous metastases remain a challenge to the oncologist. Combination chemotherapy is not yet uniformly accepted as superior to single doxorubicin. The results presented here, on a combination of the most effective single agents, are encouraging. Moreover, the regimen was well tolerated.

Tumour markers would be especially valuable in assessing prognosis, for early diagnosis of recurrence and in early response evaluation during treatment of advanced STS with chemotherapy. PIIINP may meet some of these demands. The prognostic value of the pretreatment value of PIIINP in localized, as well as extensive STS, is both theoretically and practically interesting. The increased levels of PIIINP in these various settings probably reflect different mechanisms. The development of markers of collagen type III degradation will further clarify many of the questions here raised. However, after, or during treatment PIIINP seems to be of little value.

January 1993

Radiation response of squamous cell carcinoma—In vitro studies with cell lines in a 96-well plate clonogenic assay

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The present study was undertaken to determine the variability of the inherent radiation sensitivity of squamous cell carcinoma (SCC) cell lines derived mainly from tumors of the head and neck region, and to investigate the influence of the nuclear DNA content, proliferation rate and sublethal damage repair (SLDR) capacity on the in vitro radiation response. Furthermore, the chemosensitivity to carboplatin (CBDCA) was determined, and the radiosensitizing effect of CBDCA was studied using drug sensitive cell lines of dissimilar radiosensitivity.

The recently introduced 96-well plate clonogenic assay was tested as a method for determining radiosensitivity in a panel of 37 SCC lines. Higher plating efficiencies than previously reported with the soft-agar clonogenic assays were obtained for most of the cell lines, and the results were highly repeatable. Marked differences were observed in the inherent radiosensitivity among head and neck SCC cells. This is in line with previous results obtained with other clonogenic and nonclonogenic assays. The radiosensitivity of multiple cell lines derived from the primary and recurrent tumor or regional metastases of the same patient was similar. This indicates that the inherent radiation sensitivity is a stable cellular characteristic that is not affected by factors like tumor progression or given treatment.

When head and neck SCC cell lines as a group were compared with vulvar SCC lines, the latter were found to be significantly more radiation resistant, which is consistent with the poor clinical radiocurability of vulvar carcinomas. Still, no association between the inherent radiation sensitivity of head and neck SCC cell lines and clinical radiocurability of the donor tumors was found. These results indicate that radiocurability of SCC is determined by cellular radiation resistance, but in cases where the cells are relatively radiation sensitive, this parameter does not alone predict clinical radiation response. Thus, ranking the cellular radiation response into relatively sensitive and relatively resistant seems relevant from the clinical point of view.

The nuclear DNA content of the SCC cell lines was considerably dissimilar, and showed a significant association with the inherent radiation resistance when both head and neck cancers and vulvar carcinomas were evaluated. Among cell lines derived from head and neck SCC only, no such correlation was, however, found. The in vitro doubling time was determined from the growth curves of the cell lines, but no association was found between the doubling time and the nuclear DNA content, or between the doubling time and the inherent radiosensitivity.

The 96-well plate clonogenic assay was further modified for studies on fractionated radiation and for determining the amount of cellular SLDR capacity. Ten cell lines were chosen for the experiments on the basis of their dissimilar inherent radiation sensitivities. Dividing the radiation dose into two and three equal fractions administered at a 24 h interval caused an enhancement in survival in some, but not all, cell lines. The increase in survival was not associated with the doubling time of the cell lines, and was thus considered to reflect cellular SLDR capacity. The increase in SLDR was associated with increasing inherent radiation-sensitivity, and the two cell lines that lacked SLDR, were also the most radiation resistant. The clinical outcome of the patients from whom the sensitive cell lines were derived, was poor despite of radiotherapy. This suggests that a high repair capacity may result in low radiocurability of some inherently radiosensitive carcinomas.

Finally, the sensitivity of the SCC cell lines to CBDCA and the combined effects of CBDCA and radiation were studied by using the modified 96-well plate clonogenic assay. The CBDCA concentrations needed for 50% survival inhibition varied remarkably in 6 cell lines. No correlation was, however, found between the relative drug sensitivity and the inherent radiation sensitivity. The IC_{50} values for CBDCA in the two radiation resistant cell lines were less than half of the highest IC_{50} values in the whole series. Thus, it seems that radioresistance of SCC cells does not necessarily mean cross-resistance to CBDCA, as has been reported for CDDP. Concomitant administration of CBDCA and

single radiation doses in two of the most drug sensitive cell lines produced an additive effect. These results indicate that if toxic effects are not significantly increased by combination of these two cytotoxic agents, chemoradiotherapy may bring about improvement in the treatment of some radioresistant head and neck carcinomas.

January 1993

DNA flow cytometry and prognosis of metastatic melanoma

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The aim of this study was to investigate the value of DNA FCM in metastatic melanoma and to correlate DNA ploidy and S-phase fraction with clinicopathological features and prognosis of the patients. In addition to standard DNA flow cytometry the growth rate of tumor specimens under the mouse renal capsule was assessed.

Specimens of metastatic lesions form 117 patients treated at Helsinki University Central Hospital were collected for analysis. One third of the patients had a tumor with diploid DNA content. Tetraploidy was seen in 7% of the patients. Aneuploid DNA indices were evenly distributed over the range from 0.66 to 2.69. Multiple abnormal clones were detected in 15%. This heterogeneity of melanoma metastases was further emphasized by the observation of DNA ploidy heterogeneity between different metastases from the same patients. Metastases originating from melanoma of a lower extremity were more frequently aneuploid than metastases originating from all the other sites.

The mean SPF of diploid tumors was significantly higher than that of aneuploid tumors. Furthermore, SPF of regional lymph node metastases and skin metastases was significantly lower than that of distant metastases.

DNA ploidy proved to be a stage-independent prognostic factor in metastatic melanoma. In contrast to expectations based on DNA FCM data from primary melanomas, DNA aneuploidy turned out to be a favorable sign. High SPF was a highly significant adverse prognosis sign within the ploidy groups.

The prognostic accuracy of the FCM analysis was further improved by combining DNA index and SPF to define three different types of DNA histograms associated with favorable, intermediate and poor prognosis of the patients. According to a Cox multivariate analysis, the independent prognostic factors in metastastic melanoma included the DNA ploidy, SPF, tumor growth rate under the renal capsule and TNM stage. These parameters were combined to create a versatile prognostic scoring system, which can separate patients with highly different prognosis. Patients with a low score survive a median of 29 months, while high-score patients have a median survival of 11 months.

DNA flow cytometry can be used as a tool to provide prognostically significant data on metastatic melanoma. The results of the present study, especially that of DNA diploidy as an adverse prognostic sign, are somewhat conflicting with earlier assumptions and should, therefore, be confirmed in another patient population. The results, when confirmed, provide a new important approach and should be incorporated in controlled clinical trials to learn exactly how the prognostic information provided by flow cytometry could be used to assist the selection of therapy for the individual patient.

February 1993

Determination of estrogen receptors in paraffin-embedded tissue—Techniques and the value in breast cancer treatment

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It has been established that immunoenzyme and immunohistochemical assays for estrogen receptor determination in fresh tissue correlate closely with conventional steroid binding assays. This implies that their value in the management of patients with breast cancer is of the same magnitude as that of the steroid binding assays, although the direct clinical evidence is so far very limited. With the present knowledge, a change from binding assays to immunoenzyme and immunohistochemical assays for estrogen receptors can only be recommended if clinical correlations can be established and national and international quality control studies of the same kind as for laboratories performing binding assays are implemented. Immunohistochemical analysis for estrogen receptors in paraffin-embedded tissue suffers from a loss of immunoreactive ER due to tissue processing and fixation, but modifications of the immunohistochemical techniques can partly compensate for the decreased sensitivity.

The value of ER as a prognosticator in patients with early breast cancer is in general low, and significance is not achieved in most studies using multivariate analysis. ER-PAR cannot be used as a prognosticator, but ER-DCC is an independent but weak prognostic factor in selected subgroups.

ER analyses in patients with early breast cancer receiving adjuvant hormonal treatment: Meta-analyses show a significant effect of adjuvant tamoxifen in postmenopausal patients with early breast cancer. The use of ER status as a criterion for treatment allocation is attractive as a treatment benefit cannot be ruled out initially. The use of ER analyses for treatment stratification should be done with caution because at least two large-scale adjuvant trials have shown that also ER-negative patients benefit from adjuvant tamoxifen treatment. The value of ER-PAR is undetermined, but it may be of the same magnitude as ER-DCC. Further definition of the role of ER is required, and can best be obtained from controlled clinical trials in which ER is measured with high-quality ER assays. A possible shortcut may consist in a retrospective analysis of tumors from one of the larger randomized trials using a paraffin-based ER assay.

Immunohistochemical ER analyses are useful predictors of response to endocrine therapy in patients with advanced breast cancer. The predictive value seems to be of the same magnitude as that of ER-DCC assays despite differences in the material used for analysis and the differences among the criteria for interpretation of the ER-status. The value of ER-PAR appears to be equivalent to that of assays in fresh or frozen tissue.

March 1993

Localized carcinoma of the prostate—Aspects on screening, staging, and surgical treatment

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The management of localized carcinoma of the prostate is controversial. Opinions vary about whether screening is appropriate for this particular cancer, about the accuracy of pretreatment staging of the primary tumour, and about the value of potentially curable treatment such as radical prostatectomy.

In the present study screening was assessed from an organizational, medical, economical, and psychological view. 1 494 men were selected from a population of 9 026 men aged 50–69 years and allocated at random to a screening programme for prostate cancer by digital rectal examination. There were two screening rounds, that in 1987 being conducted by an urologist and a general practitioner, and that in 1990 by a general practitioner only. The remaining 7 532 men served as a control group. 78% accepted the invitation to the first screening round, and 70% to the second. In the study group 17.4 prostate cancers were diagnosed per 1 000 men, and in the control group 8.6 per 1 000 men. The screening cost of the programme was 18 000 SEK per detected cancer, and 25 700 SEK per detected cancer treated by a potentially curative method. The long term consequences were analysed using a decision analysis model, and compared with 2 other alternatives for early detection. Inter observer variation in the digital assessment by urologist and general practitioner was analysed. Contrary to the general belief that digital rectal examination is highly subjective, we found good conformity between the observations of the two categories of examiner.

In 29 patients the predictive value of fine-needle aspiration biopsy was compared with the histological findings in whole organ sections of radical prostatectomy specimens. The preoperative cytological examination predicted correctly the grade of malignancy in the whole organ sections in 59% of the cases. No overestimation of the grade occurred.

Transrectal ultrasonography was used to predict the category of the primary tumour in 59 patients, and ultrasonically guided biopsies were added in 35. Histopathological examination of the whole organ sections was compared with the ultrasonic findings in 49 cases. The accuracy of staging improved with increasing experience.

To investigate the role of radical prostatectomy a series of 182 patients was reviewed. The pathological staging was based on whole organ sections. In 109 (60%) cases the tumour was confined to the prostate or the specimen. The Gleason score was significantly higher in patients with involvement of the surgical margin or the seminal vesicles. Urinary continence was regained in 79%, but only 19% were potent after operation. In 131 patients who underwent radical prostatectomy the quality of life was studied by questionnaires. In the long term only distress due to loss of erection persisted, but the overall wellbeing after 18 months was better than before operation.

Screening for carcinoma of the prostate by digital rectal examination can be organized with a high population acceptance and at reasonable cost per detected cancer. The introduction of general screening would call for a moderate expansion of available resources. The impact of screening on mortality remains uncertain. Fine-needle aspiration biopsy predicts the grade of malignancy in most cases. Transrectal ultrasonography in combination with ultrasonically guided biopsy can facilitate the choice of treatment. The complication rate after radical prostatectomy is low, and assessment of quality of life complements knowledge concerning the clinical outcome of radical prostatectomy.

March 1993

Malignant astrocytomas—Studies in diagnosis and treatment

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We included 171 patients with malignant astrocytomas in a controlled, prospective, randomized study. All patients were given combination chemotherapy, procarbazine, vincristine, and CCNU (PVC), and half of the patients received whole-brain irradiation (RT). Median time to progression (MTP) was 21 weeks and median survival time (MST) was 53 weeks; 18% of the patients survived 2 years or longer. Patients less than 50 years of age treated with PVC and RT had significantly longer MTP (81 weeks) and MST (124 weeks). We could not demonstrate a beneficial effect of RT in patients older than 50 years of age. Age, Karnofsky index, areas of grade 2, and absence of necrosis in the tumor were significant prognostic factors. When tumor progression was diagnosed, all patients were considered for reoperation. Of 143 eligible patients 58 were reoperated and 85 were not. In univariate analyses reoperation, age, and high Karnofsky index were highly significant for longer survival after progression. A multi-variate analysis revealed no significant effect of reoperation on survival, while age, and Karnofsky index remained strong independent factors. The very liberate use of corticosteroids in brain tumor patients are questioned after our demonstration of mutual interdependence of mononuclear leukocyte adenosine diphosphate ribosylation and corticosteroid-induced immune cell dysfunction in patients with malignant astrocytomas, as this could suppress the host immune defence system. We have elaborated a successful method of transplantation of human malignant astrocytomas into the brain of normal adult rats for tumor studies. The laser-induced fluorescence spectra of malignant astrocytomas have been evaluated in animals and humans in order to develop a technique of separating tumor and surrounding normal brain during operation. The meningeomas is separated from the astrocytomas by tissue autofluorescence spectras. After utilizing the enhancing effect of haematoporphyrin derivative we could clearly identify the malignant tumors in animals but not in humans, where we presumably used too low doses of haematoporphyrin.

March 1993

Neuropeptide Y and nerve growth factor receptors in neuroblastoma and leukemia in children

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Children with acute leukemia and neural crest-derived tumors of the sympathetic nervous system, malignant neuroblastomas and benign ganglioneuromas, were studied. Neuropeptide Y and receptors for the nerve growth factor (NGF) were investigated to explore new possibilities for diagnosis, monitoring, and characterizing these diseases.

Concentrations of NPY immunoreactivity were analyzed in plasma, tumor tissues and control tissues. Children with neuroblastoma and poor outcome had elevated plasma NPY compared to an age-adjusted reference interval established from healthy controls. Children with ganglioneuroma and favorable neuroblastomas had normal plasma NPY. Serial plasma NPY monitored the clinical course in all children with neuroblastoma and proved sensitive for early detection of relapse. Renal failure may however cause falsely elevated plasma NPY. Tumor NPY was elevated in 68 of 83 primary neuroblastomas and further increased in metastases. Only 4 of 11 ganglioneuromas had higher concentrations than healthy adrenals and NPY was not detected in any of 16 Wilms' tumors. N-*myc* amplified tumors from children >2 years had low NPY concentrations reflecting an undifferentiated tumor behavior. NPY mRNA was coexpressed with low affinity NGF receptor (LNGFR) mRNA and *trk* protooncogene mRNA indicating NGF regulation of NPY expression in neuroblastoma in vivo.

Three different NPY immunoreactive molecular forms were characterized in neuroblastoma tissue. One precursor, proNPY, was present in all malignant neuroblastomas and normal adrenal tissue but not in benign ganglioneuroma or pheochromocytoma. Intact NPY (1–36) was detected in all tissues with NPY immunoreactivity and isolated and structurally characterized from one neuroblastoma tumor. One third form, a smaller homologue with intact C-terminal part indicating biological activity, was characterized in a neuroblastoma tissue.

Children with actue lymphoblastic leukemia of B-cell precursor differentiation had elevated plasma NPY with close correlation to expression of CALLA (CD10) on leukemic blasts. NPY mRNA was detected in leukemic and healthy bone marrow with Northern Blot. In situ hybridization revealed NPY mRNA in CALLA-positive lymphoblasts. In children with leukemia and lymphoma of B-cell precursor differentiation plasma NPY followed the clinical course with decreased concentrations at remission and increased at relapse. Plasma NPY correlated with favorable prognosis in leukemia.

LNGFR and *trk* mRNAs were coexpressed in neuroblastomas with favorable outcome irrespective of age or clinical stage. Only tumors coexpressing mRNAs for NGF receptors underwent spontaneous regression. LNGFR and *trk* were inversely correlated to N-*myc* amplification. Functional NGF receptors seem to be essential for neuroblastoma differentiation and regression. NPY is synthesized in neuroblastoma tumors, leukemic and healthy bone marrow, and plasma NPY is a useful marker and prognostic indicator in children with leukemia and neuroblastoma. NGF receptors are critical for neuroblastoma differentiation, and lack of functional NGF receptors predicts poor outcome irrespective of other features. The loss of NGF receptors may constitute an early critical event in tumorigenesis of advanced neuroblastomas with poor prognosis.

April 1993

Human breast carcinoma—Clinical relevance of cellular proliferation, DNA ploidy and proto-oncogene activation

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Breast cancer is the most common malignancy among women. The death rate is still high in many subgroups of patients in spite of considerable efforts to improve treatment in the primary situation as well as in advanced cases. Although some progress has been made during the last decades we still do not know how to select patients for the most optimal treatment. There is a need for basic studies in breast cancer biology and also for investigations of the clinical relevance of the results from these basic studies. Novel techniques in cell biology, cell kinetics, molecular biology and immunohistochemistry have made possible such studies of individual patients' tumours. Of special interest is the potential of new prognostic factors that may both give a deeper understanding of the breast cancer disease and in addition, hopefully, may have a clinical impact on handling patients.

The present study focuses on in vitro studies of breast carcinoma cells from patients. Various parameters have been studied and correlations to clinical parameters, including prognosis, have been performed. In principle three types of studies have been performed. In the following these are summarized and conclusions are made.

In vitro proliferation of human breast carcinoma: Two hundred and thirty-seven breast carcinomas were cultivated in soft agar according to the Courtanay-Mills soft agar method. Seventy-five percent of the tumours formed colonies, 60% formed >30 colonies. This method was found to be more optimal than the commonly used Hamburger-Salmon method. Colony formation was marginally stimulated by addition of hormones. After addition of various anticancer agents differential dose–response relationships were obtained.

Colony formation in vitro was then correlated to various clinical and histopathological parameters. No significant correlation was found and colony formation was not found to be a prognostic factor of any value in the clinical situation. The Courtenay-Mills soft agar method enables colony formation from breast carcinomas in a rather high percentage of cases. Thus, the system permits biological studies and studies of treatment efficacy, like chemotherapy and radiotherapy. However, due to a relatively low cell yield from breast carcinomas and absence of sufficient colony formation in a significant number of cases the method cannot be recommended on a routine basis for individual handling of patients. Moreover, colony formation cannot be used as a new prognostic factor in human breast carcinoma.

Flow cytometric DNA analysis in human breast carcinoma: Fresh tumour tissue from 198 primary invasive breast carcinomas was analysed by DNA flow cytometry, and ploidy and S-phase fraction were calculated. About half the tumours were found to be non-diploid. In correlation studies a higher proportion of non-diploid tumours was found among node positive patients and patients with oestrogen receptor negative tumours. The survival of patients with diploid and non-diploid tumours was not significantly different. S-phase fraction was significantly correlated to histopathological grading. Moreover, the distribution of SPF was different in diploid and non-diploid tumours. Patients with low SPF had a significantly longer survival than had patients with high SPF. SPF was found to be an additional prognostic factor next to node status and ER status. DNA ploidy was not found to be a prognostic factor in breast carcinoma patients. In contrast, S-phase fraction was significantly correlated to survival of patients and can represent an additional prognostic factor next to node status and ER status. In spite of this finding the clinical usefulness of this parameter is still questionable in handling of individual patients.

Proto-oncogenes in human breast carcinomas: Activation of the proto-oncogenes c-erbB-2, int-2 and c-myc was investigated in tumour material from breast carcioma patients at the DNA level. In addition, oncogene expression of c-erbB-2 was studied at the protein level by immunohistochemistry. c-erbB-2 was found to be amplified in 22.5% in the total material of primary tumours and in 12% of tumours from node negative patients. c-erbB-2 protein overexpression was more frequently found in metastases than in primary tumours. Amplification of c-erbB-2 was not correlated to node status, but amplification was more frequent in ER negative tumours. No significant association was found between relapse-free survival in node negative patients with c-erbB-2 amplified tumours versus patients with c-erbB-2 non-amplified tumours. The int-2 gene was found to be amplified in 8.6% of node negative tumours. A correlation to oestrogen receptor positivity was found, but not to prognosis of patients. Only one of 87 tumours was found to be amplified for c-myc.

Activation of the proto-oncogene c-erbB-2 was somewhat more frequent in metastases than in primary tumours, indicating that amplification or overexpression may be a rather late event in tumour progression. In the present study c-erbB-2 amplification/overexpression has not been shown to have any significant prognostic impact. The prognostic value of c-erbB-2 is still an unresolved question and larger studies are still needed.

April 1993

Malignant melanoma in Sweden

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Incidence rates were analyzed in all 15 376 patients diagnosed as having cutaneous malignant melanoma (CMM) in Sweden during 1960–1984. In men, age-standardized rates of CMM located on the trunk increased from 1.1 to 7.0 per 100 000. The largest increase was seen at 60–69 years of age (2.3 to 15.1). In women, CMM on the upper extremity, lower extremity and trunk increased similarly by about 3-5 times, being most marked (1.0 to 5.1) on the upper extremity at 70–79 years of age.

Age + period + cohort analyses showed that the changing rates in men with CMM on the trunk, upper extremity and lower extremity were explained by cohort effects, whereas the rates in women with CMM on the trunk and lower extremity changed similarly in most age-groups, in accordance with time-period effects.

Analyses of the mortality rates were based on 6 324 cases of CMM reported to the National Cause of Death Register in 1953–1987. In men, age-standardized rates rose from 1.1 to 4.0 per 100 000 and in women from 1.0 to 2.6. The annual percentage change decreased in men from 4.6% during 1953–1967 to 2.0% during 1978–1987 and in women from 3.7% to 0% during the same periods. Age + period + cohort analyses showed that the changes in rates were mainly due to cohort effects in men and to time-period effects in women. Forcasts of mortality rates were calculated for patients between 20–84 years of age and they were extrapolated to the year 2007. Among men, a slight increase to 7.4 (per 100 000) seemed likely, whereas among women, the rates will probably remain unchanged (3.0).

The possible association between the clinical variables of gender, age, anatomic site and period of diagnosis and relative survival (RS) from CMM was investigated in patients diagnosed as having CMM in Sweden 1960–1982. All variables were independent prognostic factors. Among women the 10-year RS was 75% (95% confidence interval 73–77%) and among men, 62% (60–64%). Patients younger than 50 years of age had a better prognosis than those older than 60 years of age ($p < 0.001$). CMM located on the scalp-neck or trunk had a worse prognosis than on other anatomic sites. The relative risk of dying of CMM, during the first 5 years of follow-up, decreased by 64% in men and by 71% in women for tumors diagnosed 1975–1979, as compared to those diagnosed 1960–1964.

Clinical and histopathological predictors of survival were further identified in a population-based sample of 498 patients diagnosed 1960–1984 in seven counties in central Sweden. The quantitatively most important prognostic factor was tumor thickness. A predictive model was estimated and based on eight variables (gender, age, time period of diagnosis, anatomic site, clinical stage, tumor thickness, ulceration and vascular invasion), a prognostic index was constructed which distinguished patients with a high risk or a low risk of dying of the disease.

CMM diagnosed during more recent periods are thinner, less frequently ulcerated and more often of the superficially-spreading histogenetic type than the tumors diagnosed 1960–1969. These changes are probably explained partly by earlier diagnosis and partly by changes in the biology of the disease.

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Chromosome aberrations in relation to malignant disease—A cytogenetic investigation

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Chromosomes were prepared from bone-marrow samples from healthy students and patients with chronic myelogenous leukaemia. The possibility of improving the methotrexate technique was analysed. By extending the incubation period before the methotrexate treatment and shortening the Colcemid and hypotonic treatment, longer chromosomes and larger areas of the mitotic figures were obtained.

To study inherited chromosomal abnormalities, a pedigree of 36 family members was constructed. Chromosome analysis and carried out in G- and C-banding preparations from peripheral blood cultures of 19 family members. Distinct heteromorphism in the chromosome 1qh region was detected in 15 of them. A correlation between heterochromatin heteromorphism and occurrence of cancer in possible.

Variations of the C-band region of chromosome no. 1 regarding band size, intrapair size asymmetry and inversion-incidence were analysed using a computer-based interactive image analysis system. Heterochromatin size asymmetry was estimated visually and determined by objective measurement of length and area of 1qh. Asymmetry indices of length and area measurements correlated well, implying that the simpler method of length measurement can be readily used.

No significant correlation was found between the size of 1qh and presence of ovarian cancer. Asymmetry of 1qh, measured objectively or estimated visually, was significantly increased in the cancer patient group.

Karyotypes of ovarian cancer patients were studied in G- and C-banded preparations from peripheral blood lymphocyte cultures in order to determine any chromosome damage that might possibly be related to chemotherapy and radiotherapy and to study asymmetry of the constitutive heterochromatin of chromosomes 1, 9 and 16. Chemotherapy was either single-drug therapy using melphalan or multidrug therapy. The incidence of chromosome structural changes and chromosome abnormalities was higher in the melphalan-treated groups than in controls. In the patients receiving combined treatment with melphalan and radiation, the rate of chromosome aberrations and structural changes increased dramatically. In new blood samples, collected after 5 years, all patients showed abnormal karyotypes. All chromosome abnormalities in the second analysis were completely unrelated to those in the first analysis. C-band heteromorphism of chromosomes 1, 9 and 16 and occurrence of inversion in chromosome no. 1 were significantly increased in the cancer patients studied.

Genetic stability was studied over 2 years of continuous cultivation by repeated flow-cytometric and cytogenetic analysis in four cells lines established from human metastatic malignant melanoma. The DNA indices ranged from 1.7 to 2.1 and were stable throughout the entire period. The same was true for the karyotypes.

The same melanoma cell lines were analysed for their sensitivity to interferon α and β in relation to the dosage of interferon genes. The anti-proliferative effect of both interferon α and β varied between the cells lines. The inhibitory effect of interferon β was clearly stronger than that of interferon α.

April 1993

Clinical and experimental studies on human leukemias with special reference to multidrug resistance

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The aim of the present study was to elucidate the role of multidrug resistance (MDR) as a mechanism of drug resistance in leukemia with the ultimate goal to improve treatment results by the addition of resistance modifiers to chemotherapy.

The accumulation and cytotoxic effect of vincristine (Vcr) was potentiated in vitro by verapamil (Ver) in blood tumor cells from patients with low grade malignant lymphoma. During oral Ver administration to patients the achieved serum levels of Ver and its metabolites were insufficient to increase Vcr accumulation. In vitro the D- and L-isomer of Ver were equally efficient and the effect was not inhibited by the addition of calcium to the incubation medium. Consequently, the effect of Ver as a resistance modifier is unrelated to its calcium channel blocking effect.

Two sublines of the human leukemic cell line K562, selected for resistance to Vcr, expressed the multidrug resistance (mdr1) gene and were cross resistant to daunorubicin (Dau) but not to etoposide. The resistance modifiers Ver and cyclosporin A restored the cellular accumulation and sensitivity to Dau and Vcr in the resistant sublines. In addition, they significantly increased the cellular accumulation and cytotoxic effect of Vcr also in the maternal P-glycoprotein negative line and in rat cardiac myocytes, which indicates that a transport mechanism affecting Vcr not related to P-glycoprotein but also influenced by Ver and cyclosporin A was present.

A subline of K562, selected for resistance to mitoxantrone (Mxn) did not overexpress the mdr1 gene. In another mdr1 gene expressing subline the cellular Mxn accumulation was only half of that in K562 cells and resistance modifiers completely restored the decreased levels. Consequently, it seems as even if Mxn does not induce mdr1 gene expression, it can be transported by P-glycoprotein. In patients with acute myelocytic leukemia (AML), receiving Mxn during remission induction treatment the concentration of Mxn was much higher in leukemic cells than in plasma. The cellular concentration was rather stable form the end of the infusion throughout the dose interval of 24 h.

Twenty of 44 patients with de novo untreated AML and 5 of 14 patients with untreated acute lymphocytic leukemia had detectable levels (0.2–2.2 transcripts per cell) of mdr1 RNA in their leukemic cells. Detectable levels of mdr1 RNA were more frequent in cells from patients with high age and the level of expression seemed to be higher in cells from patients with secondary compared to de novo AML. No relationship between mdr1 expression and the complete remission rate was found in patients with AML. Sequential analyses of leukemic cells from 18 patients revealed that treatment with combination chemotherapy did not result in an increase of mdr1 RNA levels in the remaining leukemic cells. Consequently, mdr1 gene expression in acute leukemia is more probably associated with primary rather than secondary resistance to chemotherapy.

By the use of fluorescence activated cell sorting it was found that addition of Ver increased the cellular accumulation of Dau in all of 5 leukemic cell samples with detectable mdr1 RNA levels (0.6–1.0 transcripts per cell) but also in 2 of 3 with undetectable levels. The increase was restricted to a subpopulation of cells with low Dau content in one sample. In the remaining samples the increase seemed to affect the majority of cells. Thus, by the technique used it did not appear as if mdr1 RNA levels found in leukemic cell populations reflect an expression restricted to a subclone of cells. Alternatively, the increase of Dau accumulation caused by Ver was mediated by transport mechanisms unrelated to P-glycoprotein.

May 1993

Automating the in vivo micronucleus assay in the mouse—Flow cytometric assessment of genetic damage induced by low levels of ionizing radiation and by chemicals with different induction mechanisms

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A flow-clytometric method for the automated detection and enumeration of micronuclei (MN) in mouse erthrocytes was developed. The fluorescent stains Thiazole Orange for the discrimination between polychromatic and normochromatic erythrocytes (PCEs and NCEs) and Hoechst 33342 for detection of DNA-containing micronuclei were used. The speed of analysis was ≈ 1000 cells/second and the number of cells analyzed for each sample was about $100 \times$ the recommended sample size in manual counting. Baseline frequencies of micronucleated PCEs and NCEs (MPCEs, MNCEs) were found to be approximately equal to manual scoring. Presumed MPCEs and MNCEs were verified by fluorescence microscopy of flow sorted cell populations.

Experiments with low dose rates of protracted γ-radiation were performed in order to determine the detection limit of the assay. It was found that only the dose received during the last division of the erythroblast was manifested as elevated frequencies of MN. Thus a relevant dose could be calculated by integrating the dose rate over the time needed for this last cell cycle. A relevant dose of 3 mGy gave significantly elevated frequencies of MPCEs and MNCEs. This is lower by a factor of 10 than what has previously been achieved using cytogenetic endpoints. In the region between 3 and 15 mGy of relevant dose the dose-response does not appear to deviate from linearity.

Using model clastogens and spindle poisons the DNA content of induced MN in bone marrow and peripheral blood PCEs were determined, and were found both to be characteristic for the class of genotoxic agent used, and in accordance with the presumed mechanisms of MN induction. The induced MN were further characterized by the visualization of centromeres using fluorescent in situ hybridisation on flow sorted populations of MPCEs. A good correlation between the DNA content of MN and the percentage of centromere-containing MN was found. It was concluded that the DNA content of MN was a good indicator of spindle damaging action.

May 1993

Anti-trophoblast antibodies

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Monoclonal anti-trophoblast antibodies were obtained by intrasplenic and in vitro immunizations using in vitro grown mouse egg-cylinders as the immunogen. The cell subpopulations and setup of cell surface molecules presented by in vivo egg-cylinder trophoblast cells were found to present characteristics similar to those of in vivo trophoblast cells of the same development stage.

Screening of antibody supernatants was performed against in vitro egg-cylinders (the immunogen) immobilized on nitro-cellulose papers and air dried. Positive antitrophoblast antibodies were characterized by their pre- and post-implantation stage specificity, tissue specificity and by functional effects in vitro. Immunohistochemical staining was performed using biotinylated chicken antibodies as secondary antibodies, since they had greater activity and

gave a lower background compared with antibodies of mammalian origin. Several antibodies were specific for the adhesive/invasive trophoblast cells of implanting embryos. Some antibodies were cell specific for a certain type of trophoblast cell in the mouse placenta. A few antibodies were tissue specific for the trophoblast. One antibody inhibited the in vitro growth of mouse blastocysts.

Cross-reactivity to human first and third trimester trophoblast cells was demonstrated for 20 antibodies some of which labelled only one specific type of trophoblast cell while others were tissue specific for trophoblast cells. Some of these specificities have not been demonstrated previously by antibodies.

Two types of trophoblast tumour, the non-metastatic hydatid mole and the metastatic choriocarcinoma, contained epitopes for some of the antibodies that labelled trophoblast cells of the normal placenta. Some antibodies labelled only hydatid moles, while others labelled only choriocarcinomas. By combining antibodies of different specificities, it is possible to distinguish between trophoblast cells of normal placentas, hydatid moles and choriocarcinomas.

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Immunization against polyoma virus induced tumors with synthetic peptides

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The immune response to polyoma virus induced tumors was studied. Polyoma virus is highly oncogenic in newborn and immunodeficient rodents. Animals with an intact immune system are resistant to tumor development. This is due to an efficient T cell mediated rejection of polyoma virus transformed cells expressing polyoma virus tumor specific transplantation antigens (TSTA). The aim of the present work was to study the nature of polyoma TSTA and the T cell subsets involved in the rejection of polyoma tumors. In polyoma virus induced tumors, three intracellular T angigens are expressed; large T(LT), middle T (MT) and small T (ST). In this study, it was shown that purified MT and ST had a direct immunizing capacity against polyoma tumors. Immunizing rats with cells expressing different parts of the T antigens located a poloma epitope to the 113 N-terminal amino acids common to MT and ST. Later studies by others demonstrated that intracellular proteins were presented to T cells in the form of short peptides in the context of MHC molecules. This led us to explore the possibility to immunize mice against polyoma tumors with synthetic peptides derived from the polyoma T antigens. Peptides from LT, MT and ST were found to induce a certain protection against tumor outgrowth, indicating that all three T antigens possess immunogenic epitodes. Tumors of different MHC background were affected by different peptides. Some peptides with an ability to immunize against polyoma tumors of more than one MHC haplotype were also identified. One of these, MT162-176, consisted of at least two epitopes. Some of the peptides used for immunization could bind to MHC class I in the RMA-S peptide binding assay. Further, three out of five peptides studied were recognized in the macrophage migration inhibition test by peritoneal exudate cells from polyoma virus immunized mice. In general, these peptides did not overlap with those inducing tumor reduction. Finally, to assess which T cell subsets were responsible for the rejection of polyoma tumors in virus immunized mice, monoclonal antibodies depleting T cells of the CD4 + or CD8 + subset in vivo were used. Both CD4 + and CD8 + T cells were shown to be crucial for tumor rejection.

May 1993

Neuronal differentiation of human neuroblastoma cells—Protein tyrosine kinases, protein synthesis and mRNA in growth cones

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The human neuroblastoma cell line SH-SY5Y has been used as a model system for studying neuronal differentiation in vitro. In the presence of 12-O-tetradecanoylphorbol-13-acetate (TPA) and serum the cells differentiate into a neuronal phenotype. Under serum free conditions TPA induces a poor morphological and functional differentiation. In this study it was shown that insulin-like growth factor I in combination with TPA potentiated the neuronal differentiation of the SH-SY5Y cells under serum-free conditions. This was judged by morphology and the induction of the neuronal markers, neuropeptide tyrosine and growth-associated protein 43 (GAP-43). The tyrosine specific kinases $pp60^{c\text{-}src}$ and $pp60^{c\text{-}srcN}$ ($pp60^{src}$) have implicated roles in neuronal differentiation. In differentiating SH-SY5Y cells the $pp60^{src}$ kinase activity was increased at the same time period as the cells formed growth cones and the level of GAP-43 mRNA was increased. A fractionation method was established for the isolation of growth cones and cell bodies from differentiating SH-SY5Y cells. The two fractions were characterised ultrastructurally and by biochemical methods, and then used for studying the subcellular localization of $pp60^{src}$ and two *src*-related tyrosine kinases, $pp59^{fyn}$ and $pp62^{c\text{-}yes}$. They were all slightly enriched and activated in the growth cones as compared to the levels in the cell bodies. However, only $pp60^{src}$ was found in complex with a 38 kDa protein. The 38 kDa protein was detected via its phosphorylation in a $pp60^{src}$zimmunocomplex kinase assay and the phosphorylated protein was almost exclusively found in the growth cone prepartion. These findings suggest that the closely related tyrosine kinases have different substrates and may mediate different cellular responses in the growth cone. The ultrastructural characterisation of the SH-SY5Y growth cones showed that they contained ribosomes. By metabolic labelling of isolated growth cones local protein synthesis was shown, and Northern blot analysis revealed that the growth cones contained selective mRNA species. These findings show that differentiating SH-SY5Y cells can be used as an in vitro system for studying growth cone constituents and functions.

May 1993

Adhesion molecules and MHC class I antigens in leukemia—Role in the resistance of leukemia blasts to cell-mediated cytotoxicity

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The aim of the study was to investigate and characterize the possible mechanisms of resistance of leukemic blasts to cell-mediated cytotoxicity.

The resistance of leukemic targets, form Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and leukemic cells lines, to natural killing (NK) was related in the majority of cases to the very low conjugate formation with peripheral blood lymphocytes (PBL). Activation of PBL with Interleukin-2 (IL-2) resulted in a significant increase in conjugate formation and/or in an increased killing efficiency with all tested cell lines and with the majority of AML blasts. No such effect was observed in the case of CLL.

Studies on subpopulations of lymphokine activated killer (LAK) effectors (CD3 +, CD16 +, CD56 +) showed that all of

them bound equally to both myeloid (K562, AML) and lymphoid (Raji) blasts. However, CD56 + LAK subpopulation was the most active in the interactions with K562 cell line.

A mathematical model of conjugates kinetics was formulated and validated. Several differences in the binding kinetics between myeloid and lymphoid leukemic cell lines were found.

The observed resistance to NK- and variable susceptibility to LAK-killing was not related to the expression of adhesion molecules (AM) on AML blasts. However, blocking of ICAM-1 on AML blasts inhibited binding to CD56 + LAK effectors. Moreover, in CLL lack of ICAM-1 expression could be responsible for the resistance to both NK- and LAK-killing. An additional role of NCAM-NCAM adhesion in the interactions between AML blasts and cytotoxic effectors was shown.

Blocking of MHC class I antigens on leukemic B-cells (CLL) resulted in an increased conjugate formation with both NK and LAK effectors which was followed by the increased susceptibility to lysis.

In conclusion, the resistance of leukemic cells to cell mediated cytotoxicity appears to be a complex, multi-step process in which leukemic cells can use several ways to escape immune surveillance.

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Altered adrenal steroid profile and bone characteristics in women with endometrial cancer

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The etiology of endometrial cancer (EC) includes a number of risk factors related to sex steroids. In spite of this, previous studies on endogenous steroid hormones have yielded inconclusive results, probably because of the use of poorly defined control groups. Bone is another target organ for sex steroids. Differences in bone characteristics between patients with EC and healthy control subjects would further stress endocrine dissimilarities.

Circulating levels of steroid and pituitary hormones, sex hormone-binding globulin (SHBG), insulin-like growth factor 1 (IGF-1) and bone mineral density (BMD) in the distal radius were measured in patients with EC, and in two control groups; patients with benign postmenopausal bleedings (hospital control subjects), and in healthy women free of gynecological symptoms (nonhospital control subjects).

The incidence of the first hip fracture was estimated in a population-based cohort of 2111 women with EC diagnose, who between 1965–1983 were followed up from the age of 50. This incidence was compared with the risk in the background population estimated by multiplying the person-year of observation, by the age- and calendar year-specific admission rates derived from the same population. A case-control analysis within the cohort based on medical record data were used to compare some important patient characteristics.

Patients with EC had elevated levels of weak androgens, estrogens and biologically active testosterone, as well as elevated levels of adrenal C21 steroids including cortisol. They also had higher BMD, even in subjects above 60 years of age. The differences could not be explained by anthropometric data. No difference was found in IGF-1 levels, but since IGF-1 carrier proteins were not measured, differences in that respect still are possible. The previously often used hospital control subjects differed both endocrinologically and in BMD from the healthy control subjects and resembled patients with EC in these respects.

Women with EC had a 30–40% reduction of the risk of a first hip fracture, compared to the risk in the underlying population, irrespective of age at tumor diagnosis. Higher weight was associated with lower hip fracture incidence.

The altered endocrine situation n patients with EC characterised, by both hyperandrogenicity and hyperestrogenicity, probably being a result of an increased "adrenal drive", may be an underlying reason for their hormone dependent cancer, as well as their higher BMD and the lower fracture incidence. Patients with benign post-menopausal bleedings are disqualified as control subjects in endocrine studies.

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Radiological aspects of gamma knife radiosurgery for arteriovenous malformations and other non-tumoural disorders of the brain

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The aims of the thesis were to investigate stereotaxic procedures in radiosurgery for cerebral arteriovenous malformations (AVMs) and radiation effects of single session high-dose irradiation delivered by gamma knife on the human brain. Investigation of gamma knife radiosurgery in 1 464 patients constitutes the data base of this thesis.

High quality stereotaxic angiography is the gold standard targeting imaging in radiosurgery for cerebral AVMs, particularly for small AVMs or residual AVMs after other treatments. For medium and large size AVMs, stereotaxic MR techniques can improve targeting precision and decrease irradiation volume as compared to stereotaxic angiography in selected cases provided that proper pulse sequences are used. Combined treatments, where embolization precedes radiosurgery, can improve amenability of the treatment for large AVMs. This is on condition that the partially embolized nidi are well delineated and the volume of the residual nidi has been decreased to a level where an optimum irradiation can be safely prescribed.

Radiologically, adverse radiation effects (ARE) of gamma knife radiosurgery for cerebral AVMs are observed in 16% (131/816) of the patients. The ARE are observed as a focal low attenuation on CT or as a focal high signal on MR image without enhancement in 47% (61/131), and as a peripheral or homogeneous enhancing lesion in 48% (63/131). MR imaging is more sensitive than CT in detecting the ARE. 91% of the ARE are observed within 18 months after radiosurgery and 89% are seen to regress them, i.e. 3%, are the symptoms permanent. The risk of ARE in radiosurgery for venous angiomas is higher as compared to AVMs. Other mechanisms have probably been employed.

In gamma capsulotomy, the necrotic lesions and reaction volumes created by using multiple isocentres of 4 mm collimators are less predictable as compared to that by single isocentre. Volume effects and depreciation of the steep isodose gradient are hypothesised and the leading factors of the inconsistency.

Based on the in vivo assessment of the radiation effects observed on the basically normal human brain it is concluded that irradiation volume is strongly related to the radiation effects and is one of the important considerations in decision making for radiosurgery. Volume of brain tissue exposed to irradiation could be minimised and precision of targeting could be maximised provided that a proper steoreotaxic imaging is used. The advantage derived from the imaging and treatment strategies are that

the amenability of radiosurgery for AVMs can be increased on the one hand and the risk of ARE derived from the irradiation can be decreased on the other.

June 1993

Fibroblastic, myofibroblastic and myogenic spindle-cell lesions of a reactive and neoplastic nature

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Fibroblastic, myofibroblastic and myogenic spindle-cell lesions representing both benign and malignant tumors, as well as lesions of a non-neoplastic, reactive nature, have been analyzed in order to increase our knowledge and understanding of their cellular differentiation and biologic behavior and to establish diagnostic criteria. The series comprised 10 leiomyosarcomas and one leiomyoma from large veins, 9 angiomyomas, 10 cutaneous and 7 subcutaneous leiomyosarcomas, 3 infantile spindle-cell sarcomas, designated infantile rhabdomyofibrosarcoma by us, 6 infantile fibrosarcomas, 7 proliferative myositis and fasciitis and 12 pseudomalignant spindle-cell proliferations of the urinary bladder.

The lesions have been analyzed using the following methods; 1) clinical and follow-up methods; 2) light- and electron-microscopy; 3) immunocytochemical analysis with antibodies to different intermediate and thin cytofilaments and other cytoplasmic, nuclear and stromal antigens; 4) static DNA analysis using cytometric assessments in image analysis systems based on a video-CCD camera; 5) cell culture studies and cytogenetic analyses.

The expression of thin and intermediate cytofilament sin benign and malignant smooth-muscle tumors revealed generally, but not always, an immunophenotypic fidelity to that of the supposed smooth muscle of origin. The intermediate filament desmin could be detected in all the formaldehyde-fixed benign and malignant smooth-muscle tumors if a battery of different monoclonal anti-desmin antibodies was used. The demonstration of muscle-specfic cytofilaments (desmin and smooth-muscle-specific actin) is a valuable complement to the light- and electron-microscopic examination in the diagnosis of leiomyosarcoma.

A hitherto unknown type of highly-malignant infantile spindle-cell sarcoma has been defined. The tumor is given the descriptive name infantile rhabdomyofibrosarcoma since the tumor cells reveal fibroblastic, myofibroblastic as well as rhabdomyoblastic features. The results indicate that infantile rhabdomyofibrosarcoma is distinguished from classic infantile fibrosarcoma and also from described types of rhabdomyosarcoma in terms of clinical presentation and biologic behavior, light- and electron-microscopic appearance, immunocytochemical properties and cytogenetic characteristics.

The pseudosarcomas proliferative myositis and fasciitis contain two different cell populations: fibroblast-myofibroblast-like cells and ganglion-cell-like cells. The two cell types can be distinguished light- and electron-microscopically, immunocytochemically as well as in terms of DNA content. The fine-needle aspiration cytology is considered diagnostic. The ultrastructural appearance and the absence of immunoreactivity for desmin help to distinguish them from leiomyosarcoma and rhabdomyosarcoma.

Pseudomalignant spindle-cell proliferations in the urinary bladder can appear spontaneously, subsequent to or simultaneously with various types of bladder mucosa damage, such as chronic and cellular DNA content help to distinguish these lesions from malignant spindle-cell tumors.

An awareness of the immunophenotypic diversity, including the occasional aberrant expression of cytokeratins, in various types of reactive spindle-cell lesion of soft tissues and mucous membranes and in smooth muscle tumors is of importance in order to avoid an erroneous interpretation.

June 1993

Tumour marker CA 50 in pancreatic cancer

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CA 50 was studied in totally 512 consecutive and prospective patients with symptoms and signs indicative of pancreatic disease. The following results were obtained: 1) Comparing 3 different CA 50 assays (RIA, IRMA and DELFIA), DELFIA showed the highest precision, the widest dynamic range 0-300 U/ml and was easier to perform. The test result for an individual sample differed in some cases between the assays. All tests had a sensitivity of 97–100% but higher cut-off levels were needed for IRMA and DELFIA to achieve satsifactory specificity and positive prediction. 2) CA 50, CA 19z9 and CA 195 showed better diagnostic properties compared to CEA and TATI. Combination of markers did not give any advangates, except in a few patients using TATI. 3) The differential capacity of CA 50, CA 19-9 and CA 195 between pancreatic cancer, chronic pancreatitis and other benign biliopancreatic disorders was high and comparable. 4) The diagnostic ability of CA 50 alone was comparable to that of symptoms and signs. CA 50 was raised in 343 patients and 234 (68%) had some typ of malignancy, 175 in the pancreas. In 28 of 42 clinically misjudged cases CA 50 alone indicated the proper diagnosis. 5) The importance of well defined inclusion criterias and the influence of included diseases on the performance parameters was studied, as well as different cut-off levels. A sensitivity of 96% for pancreatic cancer was found, 88% if all cancers were included. Rising the cut-off level 1–3 times gave a better balance between the parameters. The desired function of the marker used must be defined since it is difficult to achieve acceptable rates for all parameters at the same time in all situations. 6) The Lewis phenotype was found not to influence the serum expression of CA-19-9, which showed almost identical levels and frequencies compared with CA 50. 7) CA 50 in plasma correlated to the size of the primary cancer in patients with liver metastases, but not in the group lacking. The CA 50 levels were significantly higher in patients with liver metastases. 8) Cholestasis influenced to some degree the serum levels of CA 50 and CA 242, which correlated to bilirubin in all patients taken together but not in the malignant group. Both markers were reduced parallel to decompression of the cholestasis in the benign but were unchanged in the pancreatic cancer cases.

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Endometrial carcinoma—steroid hormones and receptors in relation to proliferation

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The significance of the hormonal milieu for endometrial changes is as well-known as its link with endometrial carcinoma. Unopposed oestradiol treatment is shown to increase the incidence for this cancer. Obesity leads to elevated levels of oestrogens and is a risk factor for endometrial carcinoma. An association between high tumour proliferation and prognosis is a general feature of human cancer. Tumour growth can be expressed as

proliferation rate and flow cytometry (FCM) is a sensitive and reproducable method to estimate S-phase fraction (SPF) and ploidy level. Both parameters have been shown to correlate with prognosis. Sex steroid hormone levels were analysed together with clinical parameters, SPF, and receptors in established endometrial carcinoma.

The study consisted of postmenopausal women with endometrial adenocarcinoma. Hormones were analysed in 127 patients, 99 were analysed for FCM and 60 for oestrogena nd progesterone receptors. RIA technique was used for hormone assay of oestrone, oestradiol, progesterone, androstenedione and testosterone plasma levels. The receptors were analysed with an immunohistochemical method, and SPF and ploidy level by flow cytometry.

A wide range of oestrogen concentrations was found. Some patients had levels comparable to fertile women. Strong correlations were found between body mass index, weight and depth of uterine cavity. No relations were found between receptors and SPF, apart from oestrogen-receptor positive tumours having a lower SPF when compared with receptor negative tumours. The influence of oestradiol on tumour proliferation expressed as SPF was ambiguous. SPF was increased with higher oestradiol levels in the group of peri-diploid, well-differentiated tumours, while a negative correlation was found for the peri-diploid, moderately differentiated tumours. The aneuploid and poorly differentiated tumours had a high SPF regardless of oestradiol concentration. The association between progesterone concentration and SPF was of a more general nature. Progesterone above a threshold level was related to a lower SPF in well-differentiated and moderately differentiated tumours. Thus endogenous progesterone seems to play a role in controlling the tumour's proliferation activity, in contrast to oestradiol, that had a role which did not appear to relate to proliferation activity in any specific direction. The only stimulative association was seen in well-differentiated tumours, but SPF was still below the mean value for all diploid tumours.

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